

1 **Platelets as crucial partners for tumor metastasis: from mechanistic aspects to**
2 **pharmacological targeting**

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4 *Platelet-cancer cell crosstalk*

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1 **Abstract**

2 Platelets are anucleated cells that circulate in the blood as sentinels of tissue integrity. In fact, they
3 are rich in a plethora of proteins and other factors stored in different granules which they selectively
4 release upon stimulation. Moreover, platelets synthesize a vast number of lipids and release various
5 types of vesicles, including exosomes which are rich in genetic material. Platelets possess a central
6 function to interact with other cell-types, including inflammatory cells and cancer cells. Recent
7 findings have enlightened the capacity of platelets to induce changes in the phenotype of cancer
8 cells which acquire invasiveness thus enhancing their metastatic potential. Thus, it has been
9 hypothesized that targeting the platelet may represent a novel strategy to prevent the development
10 and progression of cancer. This is supported by the efficacy of the antiplatelet agent low-dose
11 aspirin. Studies are ongoing to verify whether other antiplatelet agents share the anticancer
12 effectiveness of aspirin.

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19 **Keywords:**

20 Platelets; cancer cells; metastasis; aspirin; antiplatelet agents; prostaglandin E₂; epithelial-
21 mesenchymal-transitions

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1 Abbreviations

2	AA	Arachidonic acid
3	ADP	Adenosine diphosphate
4	ATP	Adenosine triphosphate
5	ATX	Autotaxin
6	Bcl-3	B-cell lymphoma 3-encoded protein
7	cAMP	Cyclic adenosine monophosphate
8	CD40L	CD40 ligand
9	CLEC	C-type lectin-like receptor
10	COX	Cyclooxygenase
11	CP	Cancer procoagulant
12	cPGES	Cytosolic PGE synthase
13	CRC	Colorectal cancer
14	CRP	Collagen-related peptide
15	EGF	Epidermal growth factor
16	EMT	Epithelial- Mesenchymal Transition
17	FA	Fatty acid
18	FACL	Fatty acid-CoA ligase
19	FcγRIIa	Fcγreceptor IIa
20	GP	Glycoprotein
21	GPCR	G-protein coupled receptor
22	HETE	Hydroxy-eicosatetraenoic acid
23	HIF	Hypoxia-induced factor
24	HPETE	Hydroperoxy-eicosatetraenoic acid
25	IL	Interleukin
26	IP	Prostaglandin I ₂ receptor
27	ITAM	Immunoreceptor tyrosine-based activation motif
28	LMWH	Low-molecular weight heparin
29	LOX	Lipoxygenase
30	LPA	Lysophosphatidic acid
31	mPGES	microsomal PGE synthase
32	MPs	Microparticles
33	NK	Natural killer
34	NSAID	Nonsteroidal anti-inflammatory drug
35	OxPLs	Oxidized phospholipids
36	PAR	Protease-activated receptor
37	PC	Phosphatidylcholine
38	PDGF	Platelet-derived growth factor
39	PE	Phosphatidylethanolamine
40	PF-4	Platelet factor 4
41	PG	Prostaglandin
42	PI3K	Phosphoinositide 3-kinase
43	PL	Phospholipid
44	PLA	Phospholipase A
45	pS6	S6 protein
46	PSGL-1	P-selectin glycoprotein ligand-1
47	RCT	Randomized clinical trial
48	RGD	Arginylglycylaspartic acid
49	RhoGEF	RhoGTPase nucleotide exchange factor
50	RNA	Ribonucleic acid

1	Syk	Spleen tyrosine kinase
2	TF	Tissue factor
3	TGFβ	Transforming growth factor β
4	TLR	Toll-like receptor
5	TP	Thromboxane A ₂ receptor
6	TX	Thromboxane
7	TXAS	Thromboxane A ₂ synthase
8	VASP	Vasodilator-stimulated phosphoprotein
9	VEGF	Vascular endothelial growth factor
10	VWF	Von Willebrand factor

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1 **Introduction**

2 Cancer is a complex disease and represents a major health problem worldwide due to its growing
3 incidence in the general population [1]. This phenomenon is dependent on several reasons,
4 including the limited knowledge on the mechanisms involved in the initial events of cancer
5 development. This is a significant deficiency which contributes to the development of inadequate
6 preventive strategies.

7 World Cancer Report shows that action on smoking, diet, and infections can prevent one-third of
8 cancers [2]. Interestingly, smoking and Western lifestyle resulting in an overall energy imbalance
9 due to a highly caloric diet, rich in fat, combined with moderate physical activity, are also risk
10 factors for the development of cardiovascular disease [3]. Another significant finding which has
11 enlightened the similarities of cancer and heart disease is that the administration of the antiplatelet
12 agent low-dose aspirin for some years is associated with a reduced incidence and mortality due to
13 cancer, in particular, colorectal cancer (CRC), at long-term follow-up [4]. Collectively, this
14 information has laid the basis for the hypothesis that platelet activation in response to tissue damage
15 is a significant contributor to early events in tumorigenesis [5]. In fact, platelets, once activated,
16 play a central role in cell-cell communication through the plethora of soluble factors and
17 microvesicles, rich in genetic material, which they release [6]. Importantly, in cancer patients,
18 platelets can present a different repertoire of proteins, mRNAs and microRNAs stored in their
19 granules due to the capacity of platelets to uptake different molecules present in the environment
20 (including plasma circulation) [7, 8].

21 The activation of platelets is now recognized as a central event linking tissue damage to the
22 development of chronic inflammation [9]. Thus, activated platelets may contribute to the formation
23 of the tumor microenvironment which participates in multistep tumorigenesis [10, 11]. In fact, it is
24 noteworthy that tumors are not a collection of relatively homogeneous cancer cells, but instead, they
25 are as complex organs constituted by individual specialized cell types scattered in the tumor
26 microenvironment [12]. The new information opens the way to novel strategies to restrain cancer

1 development by affecting chronic inflammation and possibly platelet activation. The
2 chemopreventive data of low-dose aspirin against CRC in clinical studies were recently found
3 appropriate by the U.S. Preventive Services Task Force which recommends the use of the drug for
4 primary prevention of cardiovascular disease and CRC [13].

5 The development of tumor metastases plays a significant contribution to the death of cancer
6 patients. Thus, the prevention of an initial metastasis in high-risk patients and new metastases in
7 patients with the limited disease are central therapeutic goals [14].

8 Clinical and experimental findings support the role of platelets in the multistep process of invasion
9 and metastasis [15]. Tumor cells begin metastasis by the invasion of the healthy tissue surrounding
10 the primary tumor; then, they enter the bloodstream, arrest at the first capillary bed encountered and
11 extravasate to colonize distant tissues [15]. The initiation of metastasis can be partly attributed to
12 the genetic heterogeneity among subpopulations of cells within primary tumors [16]. However,
13 other events should occur to induce an invasive phenotype of cancer cells that involve the interplay
14 of cancer cells with stromal cells and the environment. Importantly, cancer cells can change their
15 genetic program under the pressure of the innumerable molecules produced in tumor
16 microenvironment: (i) some cancer cells can acquire the features of a stem cell due to the epithelial
17 mesenchymal transition (EMT) phenomenon [17, 18]; (ii) cancer cells can acquire the expression
18 of some megakaryocytic genes thus promoting the coagulation cascade or the activation of
19 platelets (platelet mimicry) [19]; (iii) some cancer cells have the ability to mimic the activities
20 of endothelial cells and to participate in neovascularization (vasculogenic mimicry) [20]. These
21 events are promoted by the capacity of cancer cells to acquire novel phenotypic features which are
22 orchestrated by the direct crosstalk with cells of the stroma and platelets and/or by several
23 mediators that they release during cellular activation.

24 In this Review, we provide an overview of platelet biology and novel functions, beyond hemostasis
25 and thrombosis, which contribute to the development of cancer and its progression. We have
26 summarized the knowledge available on the anticancer effect of conventional antiplatelet agents

1 used in the setting of acute coronary syndrome. Also, we have described novel antiplatelet agents in
2 clinical development which might be potentially efficacious for the prevention and treatment of
3 cancer and metastases.

4 **Platelet biology**

5 *Platelet structure*

6 Platelets are small subcellular fragments (2-5 μm of diameter, 0.5 μm thick and 6-10 fl volume)
7 with a mean half-life of 7-10 days [21]. They arise from the cytoplasm of megakaryocytes, unique
8 hematological polyploid cells, that undergo differentiation and maturation upon the action by
9 interleukin IL-3, IL-6 and IL-11, and thrombopoietin [22]. Despite their small volume, platelets are
10 complex cellular elements; their ultra-structure analysis allows to understand the functional aspects
11 of these cells better. Platelets are made up of four structural regions. The *peripheral zone*, necessary
12 for the release of α -granular content during platelet activation [23] which consists of a glycocalyx
13 with adhesive properties, a plasmatic membrane rich in tissue factors [24] and an intricate
14 cytoskeletal regulating the shape of platelets. The *sol-gel zone*, which is the corresponding
15 cytoplasm part of the cellular fragment and has a pivotal role in providing a contracting system and
16 supporting the open canalicular system and the dense tubular system [25]. It is responsible for
17 changes in platelet morphology and in granule release [26]. The *organelle zone* which allows
18 platelets to be unique and distinct compared to other blood cells due to the presence of secretory
19 organelles, including lysosomes, dense granules, and α -granules [27] (Fig.1). Lysosomes contain
20 acid hydrolases, phospholipase, and kinases, that act as proteolytic and hydrolytic enzymes [28].
21 Dense granules are the smallest granules in the cytoplasm, with a high electron density due to the
22 presence of elevated levels of calcium, phosphate, magnesium, serotonin, adenosine triphosphate
23 (ATP) and adenosine diphosphate (ADP), that play a central role in platelet activation, aggregation
24 and thrombus formation [29]. The α -granules contain adhesive proteins, coagulation factors,
25 fibrinolytic factors, growth factor mitogens, cytokines, and chemokines. Some proteins, including

1 P-selectin and CD40 ligand (CD40L), translocate from these granules to the surface of platelet
2 plasma membrane in response to stimulation and play a role in inflammation and atherosclerosis
3 [30]. Importantly, α -granules contain a plethora of growth factors which contribute to the tissue
4 repair process. Among them, there are platelet-derived growth factor (PDGF), epidermal growth
5 factor (EGF), and vascular endothelial growth factor (VEGF) [31]. Moreover, platelet α -granules
6 contain transforming growth factor (TGF)- β , a molecule involved in cellular migration [31].
7 However, platelets release also antiangiogenic factors, including the platelet factor 4 (PF-4) [32].
8 Alpha-granules also contain β -thromboglobulin with chemotactic properties for fibroblasts and
9 inflammatory cells [33]. Finally, there is the *membrane zone*, and plasma membrane makes
10 extensive invaginations that form a network inside the platelet [23].

11 Activated platelets may also release two types of membrane vesicles (Fig.1), such as microparticles
12 (MPs) and exosomes. MPs are vesicles with a size of 0.1-1 μ m and no nucleus while exosomes
13 have a size of 40-100 nm and derive from multivesicular endosomes within the cell [34, 35]. The
14 MPs recognize the target cell due to the expression of cell surface proteins [36].

15 Despite the fact that platelets lack the genomic DNA, they are not completely silent. Platelets, in
16 fact, retain a small part of the RNA of megakaryocytes and the translation machinery. Thus, in
17 response to platelet activation, the translation of proteins with relevant biologic activities has been
18 reported [37]. In fact, thrombin-activated platelets synthesize B-cell lymphoma 3-encoded protein
19 (Bcl-3), a member of the I κ B- α family of regulatory proteins [38]. Evangelista et al. [39] have
20 shown that the complete suppression of thromboxane(TX) A_2 biosynthesis by aspirin *in vitro* was
21 recovered in response to thrombin and fibrinogen due to the occurrence of *de novo*
22 cyclooxygenase(COX)-1 synthesis in platelets. This phenomenon might interfere with the complete
23 and persistent suppression of TXA $_2$ biosynthesis by aspirin necessary for cardioprotection [40].

24 Moreover, fibrinogen via the interaction with β_3 integrin provides a signal for *de novo* synthesis of
25 P-selectin in platelets which is required for maintenance of the P-selectin content [41]. P-selectin is

1 involved in the interaction of platelets with other cells, including carcinoma cells [42], and it also
2 promotes the Th1-like immune response [43]. Tinoco et al. found that P-selectin glycoprotein
3 ligand-1 (PSGL-1) expressed on the surface of T cells induces their dysfunction (a phenomenon
4 called exhaustion) leading to the impediment to control and eliminate cancer cells [44].
5 Furthermore, platelets present a high number of mitochondria containing several copies of their
6 circular genome. The development of appropriate techniques has allowed characterizing the platelet
7 transcriptome and proteome [45, 46].

8 *Platelet lipidomics of eicosanoids*

9 In response to the stimulation of platelets by different platelet agonists, several phospholipases,
10 including cytosolic phospholipase A₂ (cPLA₂) are activated. cPLA₂ causes the hydrolysis of the sn2
11 acyl bond of glycerophospholipids to produce free fatty acids (FAs). The most significant FA
12 regarding cell signaling is arachidonic acid (AA) (Fig.2). AA is then transformed through
13 enzymatic and nonenzymatic pathways to the formation of several biologically active compounds
14 collectively known as eicosanoids [47, 48].

15 Eicosanoids comprise a wide array of lipids generated by three major enzymatic pathways: the
16 COX pathway; the lipoxygenase (LOX) pathway; and the cytochrome P-450 monooxygenase
17 pathway. Additionally, free radical peroxidation can non-enzymatically convert AA to isoprostanes
18 [47, 48].

19 In platelets, the activity of COX-1 catalyzes the conversion of AA to prostaglandin (PG) H₂ via a
20 two-step process (Fig. 2). In the first step, the COX activity introduces two molecules of oxygen to
21 free AA, generating the bicyclic peroxide intermediate PGG₂. In the second phase, the peroxidase
22 activity of COX-1 reduces PGG₂ to the unstable endoperoxide PGH₂ [49]. Then, PGH₂ is
23 metabolized to the biologically active prostanoids by the activity of different and specific
24 prostanoid synthases (Fig. 2). In the platelet, the primary product of the COX-1 pathway is
25 TXA₂, produced from PGH₂ through the action of TXA₂ synthase (TXAS). TXA₂ is a potent
26 stimulus for platelet aggregation and vascular smooth muscle cell contraction via the activation of

1 thromboxane receptors (TP) [50,51] (Fig.1). In platelets, the biosynthesis of PGE₂ from PGH₂ is
2 catalyzed by two different synthases: a cytosolic PGE synthase (cPGES) and microsomal PGE
3 synthase type-2 (mPGES-2) [52] (Fig.2). Finally, minor products of platelet COX-1 pathway are
4 PGF_{2α} [53] and PGD₂ [54] (Fig.2).

5 PGE₂ binds with similar affinity to four receptors: EP1, EP2, EP3, and EP4 [55] (Fig.1). Some
6 isoforms of the EP3 receptor have been identified. EP3 receptors have been shown to couple to Gi
7 and Gq proteins [55] (Fig.1). At low concentrations (<10⁻⁶ M), PGE₂ increases the sensitivity of
8 platelets to aggregating agents markedly via the activation of the EP3 receptor Gi signaling thus
9 leading to the inhibition of cyclic adenosine monophosphate (cAMP) formation [56] (Fig.1).

10 Platelets also express EP2 and EP4 receptors which are coupled to Gs thus inducing the increase of
11 cAMP levels; this effect might translate into the inhibition of platelet activation. This is the
12 mechanism by which the endothelial product of COX activity prostacyclin (PGI₂) inhibits platelet
13 activation via the binding to the PGI₂ receptor IP express on platelets [57] (Fig.1). However, it has
14 been shown that PGE₂ may inhibit platelet activation only at high concentrations through the
15 activation of IP receptors [56].

16 LOXs constitute a family of non-heme iron dioxygenases that produce bioactive lipids such as
17 leukotrienes, lipoxins, 12- hydroxy-eicosatetraenoic acids [HETE] and 15-HETE [58-60].

18 Three major types of LOX have been identified, 5-LOX, 12-LOX, and 15-LOX [59]. Human
19 platelet-type 12-LOX (p12-LOX) was established in the early 1970s by Hamberg and Samuelsson
20 [61] (Fig.1 and 2). The initial product formed from AA by the activity of p12-LOX is exclusively
21 the biologically active metabolite 12(S)-hydroperoxy-eicosatetraenoic acid [12(S)-HPETE] [61],
22 that upon reduction results in the production of 12(S)-HETE [62,63](Fig.2). 12(S)-HETE
23 potentiates platelet activation, thrombin generation, calcium mobilization and α-granule secretion
24 [64]. Recent work using small molecule inhibitors supports a pro-thrombotic role for 12-LOX in
25 human platelets [63]. The precise mechanism of action of 12-HETE on platelets is not completely
26 understood. Guo et al. showed that GPR31, a plasma membrane orphan G protein-coupled receptor,

1 displays high affinity for the human 12(S)-HETE [65] (Fig.1). However, further studies should be
2 performed to clarify its involvement in platelet function.

3 Recently, a new class of lipid mediators has been identified in human platelets, called oxidized
4 phospholipids (oxPLs) [66]. OxPLs were initially characterized as non-enzymatically-generated
5 species, but recent studies have indicated that these mediators are also generated through enzymatic
6 activity. In response to platelet activation, the enzymatic products of COX-1 and p12-LOX, i.e.,
7 PGE₂, PGD₂ and 12(S)-HETE can be reesterified possibly using fatty acid CoA ligase (FACL)
8 [67](Fig. 2) into membrane phospholipids thus changing the plasma membrane composition. In
9 fact, eicosanoids are attached to phosphatidylethanolamine (PE) and phosphatidylcholine (PC). In
10 activated platelets, both 12(S)-HETE and PG-containing phospholipids have been detected [66].
11 The administration of low-dose aspirin which is associated with the inhibition of COX-1 activity
12 translates into the reduction of prostanoids incorporated into membrane PLs [66]. It has been shown
13 that 12(S)-HETE-PE becomes externalized after its synthesis, suggesting that oxPLs may play a
14 role in extracellular phospholipid-dependent signaling events, like coagulation, that is a process
15 requiring the presence on the cell surface of negatively charged PE and phosphatidylserine [68].
16 Further studies are necessary to elucidate the biological functions of oxPLs in platelets completely.
17 In particular, it remains to explore the possibility that lipid oxidation influences the dynamic
18 behavior of the cell membrane.

19 LOX products are associated with carcinogenic processes such as tumor cell proliferation,
20 differentiation, angiogenesis and apoptosis [60, 69]. The exposure to 12(S)-HETE or the
21 overexpression of 12-LOX in cancer cells is associated with enhanced cell growth as well as
22 migration [70].

23 ***Major platelet receptors***

24 The platelet membrane contains many transmembrane receptors on their surface (Fig.1). Most of
25 these receptors have a direct role in hemostasis. In Figure 1, the most important pharmacological
26 tools to affect their activation are shown. Glycoprotein (GP) Ib-IX-V receptors are involved in

1 platelet adhesion and aggregation, following interaction with von Willebrand factors(VWF), stored
2 in α -granules; their activation leads to the release of both TXA₂ and ADP by platelets [71].
3 Three immunoreceptor tyrosine-based activation motif (ITAM)-coupled receptors are expressed by
4 platelets: (i)GPVI, a receptor for proteins of the extracellular matrix, such as collagen and laminin,
5 that signals via the associated ITAM-containing Fc receptor γ chain (FcR γ); (ii) Fc γ RIIA, an ITAM-
6 containing receptor for immune complexes; (iii) C-type lectin-like receptor (CLEC)-2, a receptor
7 for podoplanin, a mucin-type transmembrane protein expressed in multiple tissues [72].
8 GPVI, the first platelet collagen receptor, is indispensable in the modulation of platelet adhesion
9 and aggregation mediated by integrins [73]. When GPVI binds to collagen (Fig.1) or other ligands
10 such as the collagen-related peptide (CRP), rapidly induces the activation of the integrin GPIIb/IIIa
11 (α IIb β 3) [74]. The receptor GPIIb/IIIa contributes to the formation of platelet aggregates via the
12 binding of its primary ligand fibrinogen which enables the cross-linking of adjacent platelets [74]
13 (Fig.1). Other ligands, such as fibronectin, can mediate platelet aggregation in different
14 physiological and pathological conditions [75,76]. Plasma fibronectin is a dimer that contains two
15 tripeptides Arg-Gly-Asp (RGD) sites that could potentially cross-link adjacent platelets similarly to
16 fibrinogen. Indeed, early studies showed that plasma fibronectin might support platelet aggregation
17 [77]. However, other findings showed the inhibitory effect of fibronectin on platelet aggregation
18 [78, 79]. Recently, Reheman et al. [80] generated fibrinogen /VWF/conditional plasma fibronectin
19 triple-deficient (TKO; Cre+, Fnflox/flox, Fg/VWF^{-/-}) mice and their results show that fibronectin
20 may play dual roles in thrombosis and hemostasis. Its soluble form is inhibitory possibly for
21 competition with more potent integrin ligands such as fibrinogen [81]. In contrast, extracellular
22 matrix-like fibrils of fibronectin contribute to thrombosis and hemostasis by either self-assembly or
23 covalent interaction with fibrin or other matrix proteins [80, 82,83].

1 FcγRIIA is best known for its role in immune-mediated thrombocytopenia and thrombosis.
2 Moreover, FcγRIIA plays a major role in the outside-in signaling of integrin αIIbβ3 and thus
3 contributes to thrombus stabilization at sites of vascular injury [84] (Fig.3).
4 In the family of G-protein coupled receptors (GPCRs), we have to mention the thrombin protease-
5 activated receptors (PAR)-1 and PAR-4, the ADP receptors P2Y1 and P2Y12 and TXA₂ receptors
6 TPs [85] (Fig.1).
7 Thrombin is a serine protease generated during the coagulation cascade involved in fibrin formation
8 and platelet aggregation and secretion. It acts through the activation of two GPCRs expressed on the
9 platelet surface, i.e., PAR-1 and PAR-4 [86, 87] (Fig.1). PARs are activated after thrombin-
10 mediated proteolytic cleavage of their N-terminal exodomain. PAR-1 is the primary human platelet
11 receptor. In fact, thrombin is 10–100-times more potent to activate PAR-1 than PAR-4. The
12 stimulation of PAR receptors increases the levels of cytosolic Ca²⁺, induces a shape change in
13 platelets, and stimulates TXA₂ production and ADP secretion [88].
14 ADP, released from dense granules of platelets, induces platelet aggregation, but it is also involved
15 in platelet granule secretion. These effects occur via the activation of the purinergic receptors [89].
16 Three different type of ADP receptors are expressed on platelets: a P2X-type ion channel–linked
17 receptor and two P2Y-type GPCRs, P2Y1 and P2Y12. The receptors coupled with G-proteins, Gq,
18 and Gi, which activate PLC and inhibit adenylyl cyclase, respectively (Fig.1). ADP-dependent
19 activation of the P2Y1 receptor leads to an increase in intracellular calcium via Gq coupling; P2Y12
20 activation translates into Gi coupling thus resulting in the inhibition of adenylyl cyclase and the
21 prevention of the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) [89]. This
22 protein has been recently identified as a microfilament- and a focal contact-associated protein
23 whose phosphorylation correlates with the inhibition of platelet function [90]. Furthermore, the
24 stimulation of P2Y12 leads to the activation of phosphoinositide 3-kinase(PI3K) and Akt kinase
25 [89].

1 Human TP receptor exists in two isoforms TP α and TP β , generated through an alternative splicing,
2 which differ only at the C-terminal tails [55]. The Gq-coupled TP subtype, TP α , and Gi-coupled
3 TP β subtype have been shown in human platelets (Fig.1). Both the TP α and TP β subtypes mediate
4 the stimulation of PLC associated with enhanced levels of intracellular inositol 1,4,5-triphosphate
5 and diacylglycerol. The formation of inositol 1,4,5-triphosphate induces an increase in the cytosolic
6 concentration of Ca²⁺, whereas the release of diacylglycerol activates PKC. In contrast, TP α and
7 TP β play an antagonist role towards the adenylate cyclase, stimulating and inhibiting it, respectively
8 [91]. Moreover, TP activation is associated with the induction of the G12/13 pathway which
9 involves the activation of RhoGTPase nucleotide exchange factors (RhoGEFs) [92] (Fig.1). Using
10 the TXA₂ mimetic U46619, it has been shown that platelet TP activation causes platelet shape
11 change and intracellular Ca²⁺ mobilization independently of secreted granule contents [93].
12 However, U46619 acts at the Gq-coupled TP receptor to cause secretion of granule contents.
13 Finally, U46619-induced platelet aggregation depends on Gi stimulation by ADP and other released
14 granule contents [93].

15

16 **Platelet functions**

17 In their resting state, platelets show a disc-shaped and circulate freely in the bloodstream. When
18 there is a vascular endothelial damage/dysfunction, platelets are activated and adhere to the injured
19 endothelium. Then, they release a plethora of growth factors and inflammatory mediators thus
20 recruiting inflammatory cells [94]. In this scenario, platelets release ADP and synthesize
21 prostanoids, mainly TXA₂ which amplify the platelet response by activating further platelets and
22 leading to the formation of platelet aggregates [94]. Although these events are a physiological
23 response to repair of damaged endothelium, the induction of a chronic inflammatory response in the
24 context of impaired vascular protective mechanisms translates into the development of
25 atherothrombosis [9]. In addition to their canonical roles, platelets show a versatile phenotype thus

1 contributing to different processes as innate and adaptive immune responses, atherosclerosis and
2 tumor metastasis [95].

3

4 ***Role of platelets in metastasis***

5 Several findings have pointed out the central role of platelets in the metastatic dissemination of
6 cancer cells. In fact, numerous clinical observations indicate a potential relationship between the
7 blood coagulation system, platelet functions, and cancer spreading via the bloodstream [15].

8 Cancer cells that reach the bloodstream can interact with platelets. The crosstalk between platelets
9 and cancer cells confers an advantage for metastatic progression through different mechanisms [15].

10 Platelet aggregates surrounding tumor cells promote their survival, protection from immune
11 elimination and the adhesion of tumor cells to the endothelium which permits the arrest and
12 extravasation of cancer cells [15].

13 However, recent findings have enlightened novel mechanisms by which platelets may promote the
14 development of tumor metastasis. In fact, platelets can induce a more malignant phenotype in
15 cancer cells characterized by enhanced migratory properties [96-98]. Platelets, through a direct
16 contact with cancer cells and the release of mediators, including PDGF, TGF- β and PGE₂, can
17 promote EMT in cancer cells [96-98] (Fig.3). This biological process consists in the capacity of
18 cancer cells to acquire mesenchymal markers (such as vimentin, fibronectin and the transcription
19 factors Twist, Snail, and Zeb) with the concomitant loss of epithelial markers (such as E-cadherin).

20 These events translate into the acquisition of a disseminating phenotype that allows tumor cells to
21 colonize distant organs [17]. In 2011, Labelle and collaborators [96] reported that platelets induce
22 EMT in murine colon and breast cancer cells through the release of TGF- β 1 and the activation of
23 TGF- β /Smad signaling. This leads to the development of a more aggressive tumor phenotype
24 (Fig.3). Recently, we have shown that the crosstalk between human platelets and human
25 adenocarcinoma cell line HT-29 leads to the induction of EMT in tumor cells [97] associated with
26 an aberrant expression of COX-2, which is a recognized marker of tumor progression [99]. These

1 events were triggered both by a direct interaction between the two cell types and by the release of
2 platelet-derived PDGF (Fig.3). In fact, using different pharmacological tools, we found that
3 platelets interact with HT29 cells through the platelet collagen receptor GPVI and the galectin-3
4 expressed on the surface of cancer cells and which contains a collagen-like domain [97] (Fig.3).
5 Thus, the use of revacept, a new antiplatelet agent which blocks the activation of platelet GPVI
6 [100] (Fig.1), caused the reduction of platelet-induced COX-2 expression in tumor cells and also the
7 changes in EMT-related genes (e.g., Zeb, Twist, vimentin, and E-cadherin) [97].
8 Recently, Guillem-Llobat et al. investigated whether platelets trigger colon cancer cells for
9 metastasis and whether pharmacological inhibition of platelet function may prevent it. Platelets
10 induced a mesenchymal-like phenotype in HT29 cells by the downregulation of E-cadherin and
11 upregulation of Twist1, enhanced cell mobility and a pro-aggregatory action on platelets [98].
12 These changes were prevented by different antiplatelet agents, aspirin (an inhibitor of COX-1), DG-
13 041 (an antagonist of the PGE₂ EP3 receptor) and ticagrelor (a P2Y₁₂ receptor antagonist) (Fig.1).
14 The authors also found that PGE₂, released from platelets, triggered the molecular events in HT29
15 cells through the activation of EP4 receptor signaling [98] (Fig.3). It was shown that the injection of
16 HT29 cells with a mesenchymal-like phenotype, into the circulation of immunodeficient mice,
17 activated platelets which released enhanced levels of TXA₂ and PGE₂ *in vivo* [98]. The
18 prothrombotic phenotype of cancer cells undergoing EMT participated to the development of
19 metastases. In fact, the administration of low-dose aspirin, which inhibited platelet activation and
20 the biosynthesis of prostanoids, was associated with reduced formation of metastases [98].
21 The effect of different anticoagulants was investigated in the platelet/cancer cell cross-talk [101]:
22 (i) heparin and low-molecular weight heparin (LMWH) which indirectly inhibit thrombin by
23 strongly catalyzing the function of antithrombin; (ii) the Xa inhibitor fondaparinux; and (iii)
24 warfarin which indirectly affects the coagulation cascade and thrombin generation by depleting the
25 active form of the vitamin K [101].

1 Breast cancer cells (MCF-7) induced the secretion of growth factors, especially VEGF (Fig. 3),
2 from platelets and this response was prevented by heparin and fondaparinux. Suppression of VEGF
3 release was not seen from platelets of the patients who were exposed to the oral anticoagulant
4 warfarin. In contrast, inhibition of VEGF release was shown in patients treated with LMWH and a
5 lower extent in patients anticoagulated with fondaparinux [101]. Altogether these results suggest a
6 thrombin-dependent mechanism in the tumor cell-mediated release of platelet angiogenic proteins
7 and platelet angiogenic response. It has been shown that human adenocarcinomas of the colon
8 (HCT-8 and LoVo) and one anaplastic murine tumor (Hut-20) cell lines can induce platelet
9 activation via the generation of thrombin. This phenomenon may participate in tumor cell-mediated
10 platelet responses [102]. Cancer cell-derived thrombin generation can depend on enhanced
11 expression of tissue factor (TF) and cancer procoagulant (CP), i.e., a cysteine protease derived from
12 a broad spectrum of malignant and embryonic (amnio-chorion) tissue [103].

13 Another study investigated the mechanisms involved in platelet secretion by the highly metastatic
14 colorectal cancer cell line Caco-2 and the prostate carcinoma cell line PC3M-luc. These cells
15 stimulated the release of platelet dense-granules (Fig.3), including the release of ADP; then, ADP
16 caused the aggregation of platelets. Mitrugno et al., [104] showed a novel essential role for the
17 platelet immune receptor Fc γ RIIa in cancer cell-induced platelet activation (Fig.3). Using
18 pharmacological tools, it was found that the inhibition of immune Fc γ RIIa abolished tumor cell-
19 induced platelet secretion and aggregation [104]. The interaction between cancer cells and platelets
20 activated Fc γ RIIa-spleen tyrosine kinase(Syk)-PLC γ signaling pathway and dense granule
21 secretion. These effects caused a further platelet activation, and secretion of granule contents, thus
22 contributing to platelet recruitment and protection of circulating tumor cells that ultimately
23 facilitates tumor cell survival in the circulation and at metastatic sites [104] (Fig.3). Thus, Fc γ RIIa
24 could be a possible target for an antimetastatic therapy.

25 Mannori et al., examined the adhesion of six colon cancer cell lines to purified recombinant E-, P-,
26 and L-selectin. Among them, LS 180, T84, and COLO 205 can bind to all three selectins. In

1 contrast, the colon cancer cell line COLO 320 interacts with P- and L-selectin, but not E-selectin.
2 HT29 cells can bind to E-selectin but not P- or L-selectin. They demonstrated that P-selectin
3 mediates adhesive interactions of some colon cancer cells with thrombin-activated platelets. This
4 interaction appeared to depend mainly on mucin-type glycoproteins expressed on the surface of
5 cancer cells [105] (Fig.3).

6 The implication of platelet receptor GPIIb/IIIa on the crosstalk between platelets and cancer cells
7 has been investigated in some studies using different cancer cell-types [106-108]. In particular,
8 Boukerche and colleagues [106] showed that melanoma cells (M3Dau) interacted with platelets by
9 the platelet receptor GPIIb/IIIa and the GPIIb/IIIa-like complex expressed on tumor cells (Fig.3).
10 Platelets promote ADP release from melanomas by interacting with the tumor surface. Then, ADP
11 induces platelet aggregation and degranulation leading to the formation of larger platelet-tumor
12 aggregates [106].

13 Recently, using a genetic animal model of megakaryocyte-restricted knockout strategy,
14 Mammadova-Bach and collaborators [109] showed that platelets interact with breast and colon
15 tumor cells through the binding of platelet integrin $\alpha 6\beta 1$ and tumor ADAM-9, a member of
16 disintegrin and metalloproteinase family (Fig.3). This interaction promoted platelet activation,
17 granule secretion, and subsequent endothelial transmigration of tumor cells, which culminated in
18 the promotion of tumor metastasis. In this study, in addition to genetic deletion of platelet $\alpha 6\beta 1$,
19 GoH3, an integrin $\alpha 6$ -blocking antibody, was used that prevented platelet-tumor cell cross-talk and
20 diminished lung metastasis in wild-type mice. This finding suggests the possibility of developing a
21 therapeutic strategy to target this integrin and interfere with tumor metastasis in cancer patients
22 [109].

23

24 *The role of platelet-derived lysophosphatidic acid (LPA) in the metastatic process*

25 Lysophosphatidic acid (LPA) is a bioactive phospholipid involved in numerous cellular responses
26 through the activation of specific GPCRs. It has been reported that platelets represent the primary

1 source of LPA identified so far. Activated platelets release a high amount of different molecular
2 species of LPA [110] which promotes platelet shape change and aggregation through the
3 stimulation of LPA receptor type 5 (LPA5) [111]. Several studies suggest that LPA plays a
4 significant role in the development of cancer [112]. In breast and ovarian cancer, LPA, produced by
5 blood platelets, is a major factor promoting bone metastases [113]. The mechanism involves the
6 activation of the LPA receptor type 1 (LPA1) expressed in tumor cells [114]. The blockage of this
7 receptor using a specific LPA1 antagonist prevented tumor cell–platelet interaction without
8 affecting normal platelet functions [114].

9 It was recently demonstrated that platelets are involved in the biosynthesis of LPA through the
10 release of Autotaxin (ATX), an LPA-producing enzyme. In fact, Leblanc and collaborators [115]
11 reported that in tumor cell-induced platelet aggregation, platelets release ATX (both as free
12 enzymes and/or bound to β_3 integrins, $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$) which hydrolyzed LPA precursors
13 (phosphatidic acid, PC, phosphatidylserine, and PE) to form LPA, and thus promoting cancer cell
14 invasion and metastasis [115]. One mechanism explaining the pro-metastatic action of LPA is the
15 induction of EMT [116, 117]. In ovarian cancer cells, LPA promoted the nuclear translocation of β -
16 catenin with the transcriptional activation of Wnt/ β -catenin target genes and hypoxia-induced
17 factor-1 α (HIF1 α) thus inducing the expression of mesenchymal marker genes [116, 117].

18 19 **Pharmacological targeting of the platelet to fight cancer metastasis**

20 The evidence supporting the hypothesis that platelet activation is involved in the development of
21 cancer and the promotion of metastasis opens the way to the possible chemopreventive use of
22 antiplatelet agents. Here, we overview the results of the anti-cancer effects obtained with
23 conventional antiplatelet agents used to prevent atherothrombosis and with novel agents in clinical
24 development.

25

26

1 ***-Low-dose aspirin***

2 The analyses of the data from cardiovascular prevention randomized clinical trials (RCTs) with
3 aspirin have shown that the use of the drug, even at the low-doses of 75-100 mg daily which target
4 mainly the platelet, reduces incidence and mortality due to CRC and other types of cancer [4, 118].
5 Interestingly, the aspirin anticancer effect was associated with the prevention of the formation of
6 distal metastases [119] (Table 1).

7 Aspirin, acetylsalicylic acid (ASA), is a member of nonsteroidal antiinflammatory drugs (NSAIDs)
8 which inhibit prostanoid biosynthesis by an irreversible inactivation of COX-1 and COX-2. This
9 occurs through the acetylation of a specific serine residue located in the cyclooxygenase active site,
10 at position 529 and 516 of COX-1 and COX-2, respectively [120-122]. Despite the short
11 pharmacological half-life (i.e., 20 min), the daily administration of aspirin at low-doses causes an
12 antiplatelet effect because of irreversible COX-1 inactivation (occurring both in the pre-systemic
13 and systemic circulation) in the anucleated platelets characterized by a low rate of protein synthesis.
14 Chronic dosing with low-dose aspirin causes a virtually complete inhibition of platelet COX-1
15 activity ($\geq 97\%$), maximal COX-1 acetylation (75%) and inhibition of platelet function throughout
16 dosing interval (24 h) [123, 124].

17 Numerous studies using biomarkers of prostanoid biosynthesis *in vivo* have shown that low-dose
18 aspirin affects profoundly platelet COX-1 activity while causing only a marginal effect on vascular
19 prostanoid biosynthesis dependent on COX-2 activity [5, 47, 123, 125]. This selective effect is due
20 to the capacity of nucleated cells to recover a functional COX-2 in the interval between aspirin
21 doses. This knowledge of the pharmacodynamics of aspirin led to hypothesize that the anticancer
22 effect of aspirin was due to its a selective inhibitory action on the platelet [5, 125, 126]. However,
23 the use of a direct biomarker of aspirin effect which consists in the assessment of the extent of the
24 acetylation of COX-1 in cells and tissues [124, 127] has allowed to detect the acetylation of COX-1
25 by aspirin on colorectal mucosa of individuals undergoing CRC screening. The effect on colorectal
26 mucosa was lower than the acetylation of platelet COX-1. However, it translated into a significant

1 inhibition of intestinal mucosal PGE₂ associated with reduced phosphorylation of the S6 protein of
2 the 40S ribosomal subunit (pS6) [127], a protein involved in the protein synthesis and cell growth
3 [128]. Thus, low-dose aspirin, in addition to inhibiting platelet function and reducing the release of
4 platelet-derived factors, may have a direct effect on the target tissue by preventing the activation of
5 pro-tumorigenic pathways [5].

6 The antimetastatic effect of aspirin was recently demonstrated *in vivo* in a mouse model of
7 hematogenous metastasis using human adenocarcinoma cell line HT29 [98] (Table 1). Guillem-
8 Llobat et al. [98] showed that the administration of low-dose aspirin, which inhibited platelet
9 activation and the biosynthesis of prostanoids, reduced the formation of lung metastases. The anti-
10 metastatic effect of low-dose aspirin involved the inhibition of (i) the prothrombotic properties of
11 cancer cells; (ii) cancer cell EMT and migratory capacity.

12 Altogether these findings allow going a step further in the interpretation of the mechanisms of
13 aspirin as an anticancer agent. This information is relevant for the selection of the appropriate dose
14 of aspirin to use for the prevention of cancer in patients.

15

16 - ***P2Y12 receptor antagonists***

17 The ADP platelet receptor P2Y12 is the target of effective antithrombotic agents, including the
18 thienopyridines (ticlopidine, clopidogrel, and prasugrel) that irreversibly inhibit the receptor and the
19 novel direct and reversible antagonists (ticagrelor, cangrelor, and elinogrel) [129].

20 Despite several lines of evidence, obtained in experimental animal models, sustain a possible
21 anticancer effect of these drugs, the proof that this strategy is effective in patients is still missing.

22 Here, we overview the results obtained by targeting P2Y12 receptor in experimental models of
23 tumorigenesis/metastasis *in vitro* and *in vivo*. Wang et al. [130] demonstrated that tumor metastases
24 are reduced in P2Y12-deficient mice. The coadministration of the antiplatelet drugs aspirin and
25 clopidogrel prevented or delayed the development of hepatocellular carcinoma and improved
26 survival in a mouse model of chronic immune-mediated hepatitis B (Table 1) [131].

1 ***-Blockage of platelet GPIIb/IIIa receptors***

2 As reported above, the involvement of platelet receptor GPIIb/IIIa in the crosstalk of platelets and a
3 melanoma cell line was found [106]. In this study, Fab fragments of the monoclonal antibody
4 MoAb (LYP18), directed against the platelet GPIIb/IIIa complex, inhibited platelet-melanoma cell
5 interactions and platelet-platelet aggregation [106] (Table 1). In a murine model of metastasis,
6 Nierodzik and colleagues [132] found that the blockage of the platelet GPIIb/IIIa receptor, using the
7 monoclonal antibody 10E5, decreased lung colonization of cancer cells (Table 1). It has been
8 reported that the oral inhibitor of GPIIb/IIIa, XV454, protected from metastasis formation in a
9 murine model of lung cancer [133] (Table 1).

10

11 ***- PAR antagonists***

12 The importance of PAR receptors in platelet function was investigated by Italiano and collaborators
13 [134] who showed that distinct populations of platelet α -granules, containing different angiogenesis
14 influencing proteins, can be differentially released. The secretion of the different sets of α -granules
15 from platelets is regulated by PAR-1 and PAR-4 activation [134]. Thus, these receptors can play a
16 crucial role in regulating angiogenesis and, in turn, modulate the processes of wound healing and
17 tumor growth [134, 135]. The involvement of PAR receptors in metastasis is shown by the results
18 of *in vivo* studies conducted in a murine model of hematogenous metastasis. Melanoma cells were
19 intravenously injected in Par4(-/-) mice, and the protection from lung metastasis was observed
20 [136]. Also, a recent study focused on the crosstalk between breast cancer cells and platelets
21 showed that heparin and fondaparinux reduced the activation of platelets by tumor cells through the
22 prevention of PAR-1 activation by thrombin (Table 1) [101].

23

24 ***-Blockage of platelet GPVI receptor***

25 As reported in the section of platelet biology, GPVI is a key platelet receptor for collagen. It is a
26 single span transmembrane receptor, with two immunoglobulin domains associated with the FcR- γ

1 chain containing the ITAM subunit. The involvement of this platelet receptor in metastasis was
2 shown by the results of studies conducted *in vivo* and *in vitro*. In a murine model of metastasis,
3 using a Lewis lung carcinoma (D121) or melanoma (B16F10.1) cell line, an approximately 50%
4 reduction in the number of visible tumor foci was found in GPVI-deficient mice versus control
5 C57BL/6J mice [137].

6 Inhibition of GPVI-mediated platelet activation can be achieved both by anti-GPVI antibodies and
7 by the soluble GPVI receptor revacept, a dimeric soluble GPVI-Fc fusion protein, which inhibits
8 platelet aggregation without altering general hemostasis when administered to humans [100]. The
9 results of studies performed *in vitro* showed that the exposure of HT29 colon cancer cells to
10 revacept prevented the induction of COX-2 and the expression of EMT markers induced by the
11 interaction with platelets [97]. Revacept interfered with the interaction of platelet collagen receptors
12 with galectin-3 [97], a protein highly expressed in cancer cells which contains a collagen-like
13 domain [138](Table 1). The efficacy of revacept as anticancer agent effects should be verified *in*
14 *vivo* in animal models of metastasis and if confirmed this agent should be tested in patients.

16 ***-EP3 antagonists***

17 PGE₂ production, increased in inflamed atherosclerotic plaques, may participate in the activation of
18 platelets via the EP3 signaling pathway [139]. A highly selective EP3 antagonist has been
19 developed, i.e., DG-041 [140]. The administration of DG-041 to humans completely inhibited
20 platelet aggregation with no concurrent increase in bleeding time (even at high doses) [141].
21 The effect of DG-041 in platelet-HT29 cell interaction was recently evaluated [98]. DG-041
22 prevented the induction of a mesenchymal-like phenotype in cancer cells with migratory properties,
23 and these effects were associated with reduced biosynthesis of platelet TXA₂ and PGE₂ (Table 1).
24 Since platelets, but not HT29 cells, express EP3 receptors, the effects of DG-041 was dependent on
25 a specific inhibitory action on the platelet receptor [98]. This study has enlightened the role of
26 platelet-derived PGE₂ on the induction of a metastatic phenotype in cancer cells. PGE₂ acted on

1 HT29 cells via the activation of EP4 receptors (Fig.3) [98]. PGE₂-dependent activation of cancer
2 cell EP4 was involved in the induction of EMT. However, the migratory properties of cancer cells
3 required the direct interaction of platelets with cancer cells together with the activation of EP4 by
4 PGE₂ (Fig.3) [98].

5

6 **Conclusions**

7 Platelets play an important role in the processes of hemostasis. However, new knowledge has
8 enlightened that platelets orchestrate the activation of other cells involved in tissue repair, including
9 inflammatory cells and stromal cells [135]. Moreover, platelets express and secrete many pro-
10 inflammatory molecules that serve to initiate and modulate immune responses [142, 143]. In the
11 context of cancer metastasis, the formation of circulating platelet–tumor cell aggregates protects
12 malignant cells from immune elimination by natural killer (NK) cells [144]. It is noteworthy that
13 platelets express Toll-like receptors (TLRs) [145] which participate in infectious processes through
14 the induction of an antibacterial response [146]. Platelets might also promote autoimmune diseases,
15 including multiple sclerosis [147].

16 If the platelet responses are unrestrained, they play a fundamental role in the development of
17 pathological conditions such as atherothrombosis [9, 96]. Similarly, platelets promote tumorigenesis
18 by the link existing between platelet activation and the development of chronic inflammation.
19 Moreover, platelets contribute to cancer development through their crosstalk with cancer cells.
20 Cancer cells acquire a prothrombotic phenotype, thus promoting the formation of platelet
21 aggregates which surround the cancer cells; then, the exchange of factors turns on different
22 signaling pathways which lead to the acquisition of an advantage for cancer cells in respect of
23 survival and invasiveness. Thus, the final goal of platelet-cancer cell interaction is to facilitate
24 tumor colonization to distant organs [15]. The occurrence of these pro-tumorigenic functions of
25 platelets is sustained by the efficacy of the antiplatelet agent low-dose aspirin to reduce the
26 incidence and mortality for cancer [5].

1 More basic and clinical research is necessary to develop a broad armamentarium of effective
2 antiplatelet agents with the capacity to affect the multistep process of cancer metastasis. In fact,
3 many pieces of evidence suggest that different cancer cell types have developed specific
4 mechanisms to activate platelets. Thus, effective antimetastatic treatments should involve (i) the
5 characterization of the molecular determinants of platelet-cancer cell interaction and (ii) the
6 selection of the appropriate drug which affects the specific platelet receptor. However, the efficacy
7 of low-dose aspirin as an anticancer agent through the inhibition of the biosynthesis of prostanoids,
8 suggests that the blockage of the amplification of the primary platelet response is sufficient to
9 restrain cancer progression [5].

10 There is an urgent need to perform basic and clinical research focused on the study of the role
11 played by platelet activation in the different steps of cancer development. The results of these
12 studies will allow developing novel strategies to prevent cancer and to avoid that primary cancer
13 cells metastasize through the blood vessels to various distant organs.

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24
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26

1 **Figure Legends**

2 **Fig. 1 Platelet structure, primary receptors, eicosanoid machinery, and targeted therapeutics.**

3 Platelets are involved in the release of many mediators that are stored in different granules in the
4 cytoplasm and microvesicles. A plethora of proteins are contained in α -granules; there are cell
5 adhesion proteins, blood clotting factors, growth and angiogenic factors. The plasma membrane of
6 platelets expresses several transmembrane receptors, involved in the crosstalk with other platelets
7 and different cell-types. Platelets adhere to the damaged vascular endothelium through the binding
8 of integrin receptors (GP, glycoproteins) to the extracellular matrix proteins, such as collagen and
9 VWF (von Willebrand factor). Possible strategies to inhibit the adhesion of platelets to a damaged
10 endothelium involve the use of agents that interact with collagen binding sites, such as revacept,
11 thus preventing the activation of the collagen receptors GPIa/IIa and GPVI expressed on the plasma
12 membrane of platelets. Platelet aggregation, mediated by the binding of fibrinogen or fibronectin to
13 GPIIb/IIIa on agonist-stimulated platelets, is inhibited by different antagonists of the GPIIb/IIIa
14 receptor (Abciximab, eptifibatide, and tirofiban). The activation of platelets by ADP (adenosine
15 diphosphate) is mainly affected by antagonists of the P2Y₁₂ receptor, among them, there is the pro-
16 drug clopidogrel. An important antiplatelet agent is aspirin, which irreversibly inhibits the activity
17 of cyclooxygenase(COX)-1 involved in the production of prostaglandin (PG) H₂ that is then
18 converted to the potent pro-aggregatory agent thromboxane (TX)_{A₂} by the activity of TXA₂
19 synthase (TXAS). A secondary product of COX-1-dependent pathway is PGE₂ produced by the
20 activity of different PGE synthases (PGES). TXA₂ and PGE₂ cause different platelet responses by
21 the interaction with specific receptors. Various receptor antagonists in clinical development are
22 reported. Picotamide and ridrogrel act by a dual mechanism involving the blockage of TP receptors
23 and the inhibition of TXAS. There are four different receptors for PGE₂ on the platelet surface:
24 EP₁, EP₂, EP₃ and EP₄. The stimulation of EP₃ leads to platelet activation, and specific
25 antagonists are under clinical development, including DG-041. EP₄ and EP₂ signaling may increase
26 intraplatelet cAMP (cyclic adenosine monophosphate) levels and thus possibly counteracting the

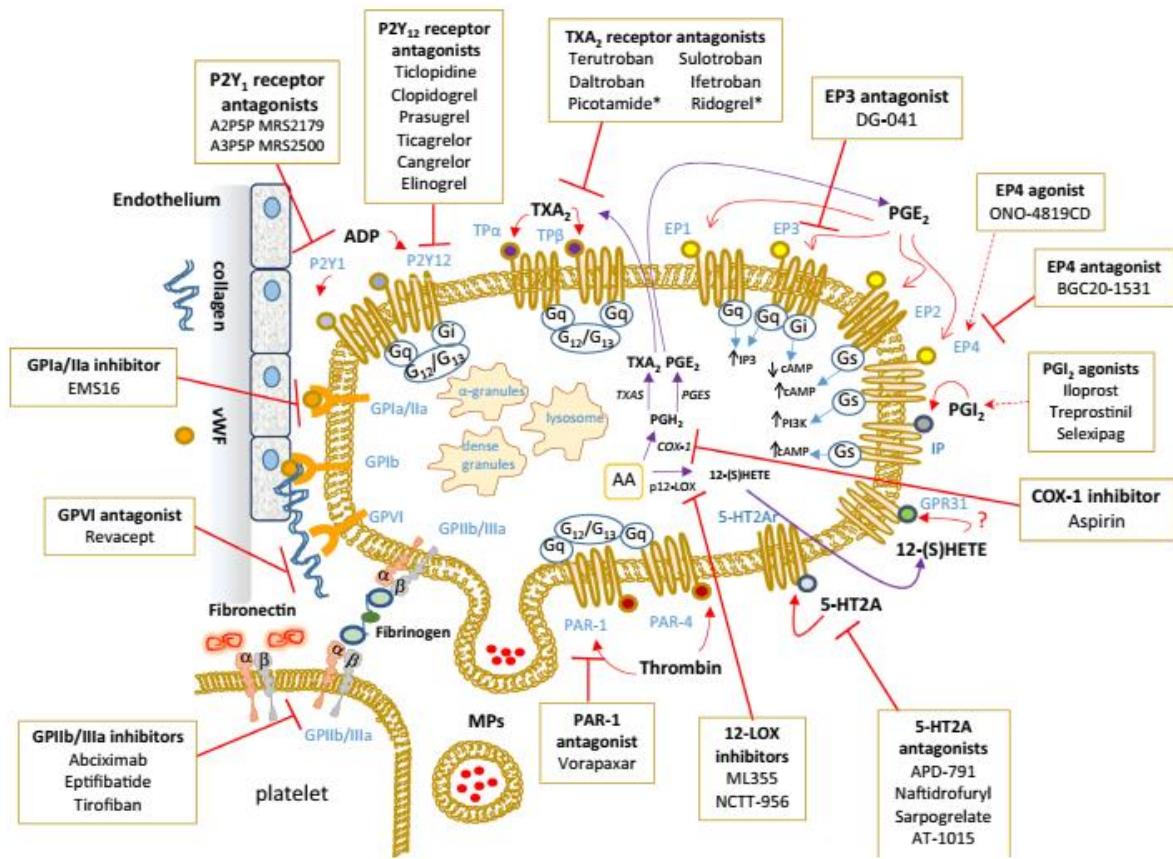
1 platelet activation by EP3. Some agonists and antagonists of these receptors have been synthesized.
2 An important receptor present on the platelet plasma membrane is the IP receptor for prostacyclin
3 (PGI₂), for which different commercially available agonists have been developed (Iloprost,
4 Treprostinil, Selexipag). Another abundant eicosanoid produced by platelets is 12(S)-HETE [12(S)-
5 hydroxyeicosatetraenoic acid] via the activity of the platelet-type lipoxygenase (p12-LOX). The
6 mechanism of action is 12-(S)HETE has not been entirely understood. Recently, it has been
7 proposed the activation of the orphan receptor GPR31 by 12-(S)HETE. The discovery of selective
8 inhibitors of 12-LOX, such as ML355, will allow enhancing our knowledge of the role played by
9 12-LOX in health and disease. Protease- activated receptors (PAR) are involved in platelet
10 activation by thrombin. There are two receptors, known as PAR-1 and PAR-4. However, PAR-1
11 possesses a higher affinity for thrombin. The PAR-1 antagonist vorapaxar was approved for clinical
12 use in 2014. New antiplatelet agents include serotonin receptor antagonists (5-HT_{2A} antagonists).

13
14 **Figure 2. Major pathways for the biosynthesis of eicosanoids in platelets.** Arachidonic acid
15 (AA), esterified in membrane phospholipids, can be released upon platelet activation by different
16 stimuli via the action of phospholipases (PLs), including the cytosolic phospholipase A₂ (cPLA₂). In
17 platelets, AA is transformed to PGH₂ by the activity of COX-1; then, PGH₂ is the substrate of
18 different synthases, thus leading to the formation of the prostanoids: TXA₂, PGE₂, PGD₂, PGF_{2α}.
19 Thromboxane synthase (TXAS), cytosolic PGE synthase (cPGES), microsomal PGE synthase-2
20 (mPGES-2), lipocalin-type prostaglandin D synthase (L-PGDS), hematopoietic prostaglandin D
21 synthase (H-PGDS) and PGF synthase (PGFS) are the downstream synthases involved in the
22 production of prostanoids. AA is also transformed to 12(S)-HETE by the activity of p12-LOX. The
23 enzyme produces 12(S)-hydroperoxy-eicosatetraenoic acid [12(S)-HPETE] which is, then,
24 converted to 12(S)-HETE by glutathione reductase (GR). PGE₂, PGD₂ and 12(S)-HETE can be
25 esterified into membrane phospholipids by the action of fatty acid CoA ligase (FACL) to produce
26 new lipid mediators, i.e. the phospholipid esterified eicosanoids.

1 **Figure 3. Molecular determinants involved in the crosstalk between platelets and cancer cells.**
2 Platelets interact with tumor cells through different receptors expressed on platelet surface [i.e.,
3 collagen receptor GPVI, P-selectin and the integrins $\alpha6\beta1$ or $\alphaIIb\beta3$, and GPIIb/IIIa]. The direct
4 interaction between the two cell-types triggers platelet activation, the secretion of platelet α -granule
5 content, i.e., growth and angiogenic factors (such as PDGF, TGF- β , and VEGF). Moreover,
6 platelets release ADP from dense granules and synthesize prostanoids, including TXA₂ and PGE₂.
7 The interaction of platelets with cancer cells causes the induction of COX-2 in cancer cells, which
8 contributes to a further increase in PGE₂ production. Aberrant PGE₂ generation is a hallmark of
9 cancer. The overexpression of COX-2 in cancer cells involves both transcriptional and
10 posttranscriptional mechanisms mediated by the release of PDGF. Among the numerous events
11 triggered by platelet-cancer cell crosstalk, there is the induction of the epithelial-mesenchymal
12 transition (EMT) phenomenon in cancer cells, which can be mediated by soluble mediators (proteins
13 and lipids) (modified from Dovizio et al., Mol Pharmacol 2013; 84:25-40 and Guillem-Llobat et al.,
14 Oncotarget 2016; 7:32462-77).

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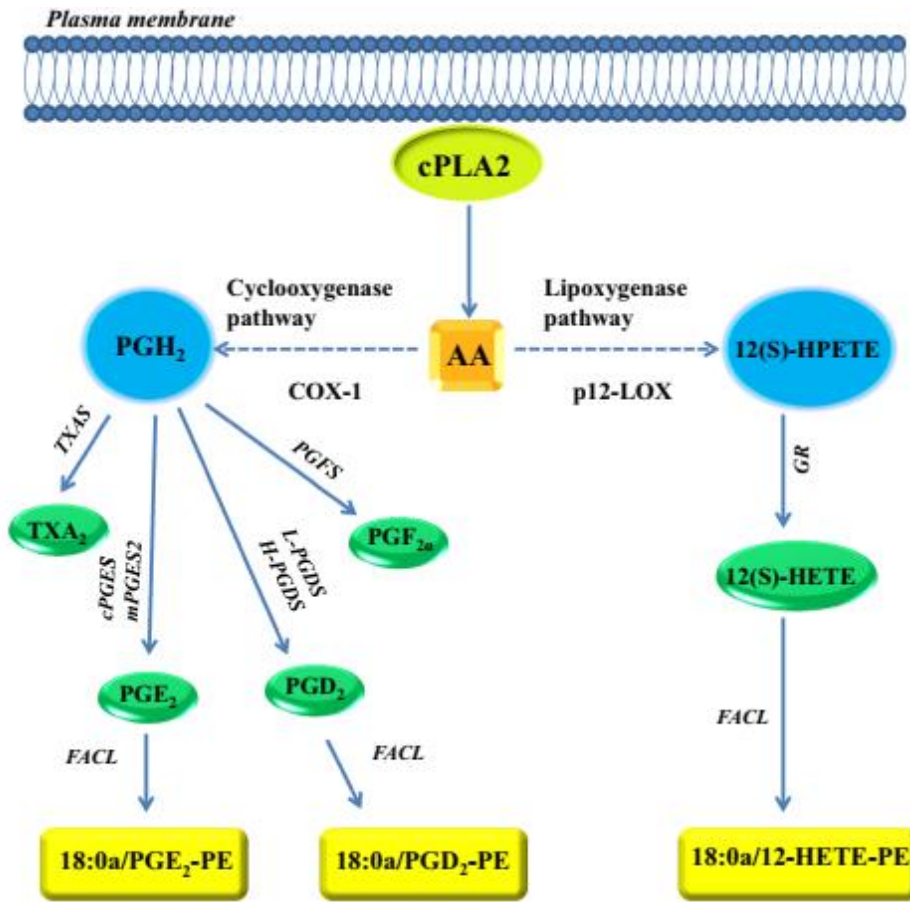
1 Fig. 1



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1 Fig. 2

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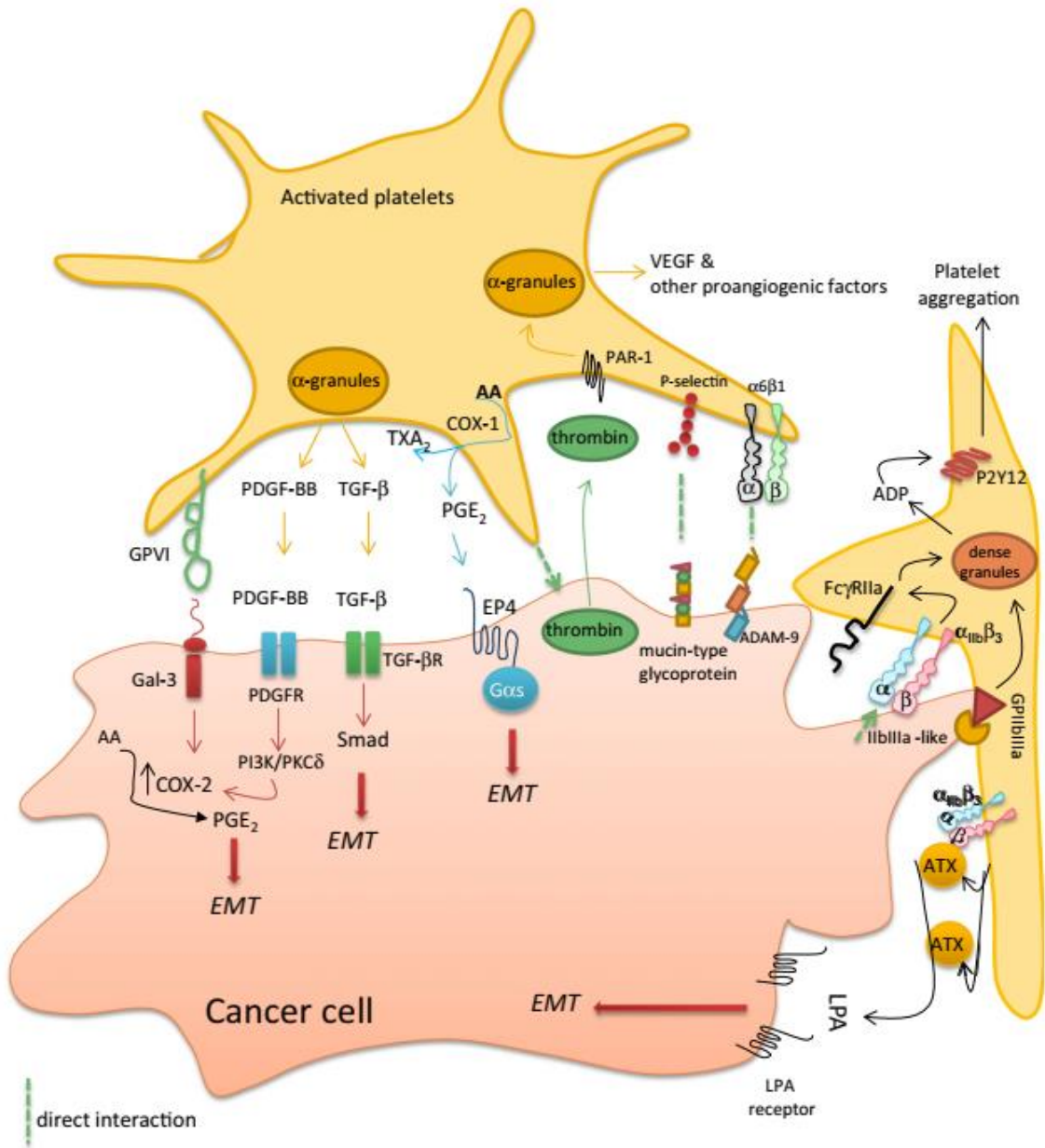
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2 **Fig. 3**



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Table 1 Experimental and clinical evidence of the anticancer effects of antithrombotic agents

Drug	Target	Effect	References
Aspirin	COX-1	Reduction of cancer incidence and death, in RCTs Anti-metastatic effect in a mouse model of hematogenous metastasis	[98, 118, 119]
Monoclonal antibody MoAb	GPIIb/IIIa receptors	Inhibition of platelet–melanoma interactions	[106]
Monoclonal antibody 10E5 and XV454	GPIIb/IIIa receptors	Reduction of lung metastases in mice	[132, 133]
Revacept	Collagen-like binding sites	Prevention of the upregulation of COX-2 and EMT in platelet–tumor cell cocultures	[97]
Heparin/fondaparinux	Indirect inhibitors of thrombin and Factor Xa	Inhibition of the activation of platelets by breast cancer cells	[101]
Clopidogrel (active metabolite)	P2Y12 receptor	Its coadministration with aspirin prevents or delays the development of hepatocarcinoma and improves survival	[131]
DG-041	Platelet EP3 receptor	Prevention of platelet-dependent induction of EMT and migration in colon cancer cells	[98]

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